

# **NIEHS-NIOSH Interagency Agreement Environmental/Occupational Immunotoxicology Studies in Humans**

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# Historical Perspective

- Established in the early 90s in general response to increased efforts to study non-cancer endpoints by the NTP
- Mission was to conduct human immunotoxicology studies in support of the NTP
- Preceded by an NTP interagency agreement (IAG) with NIOSH involving male reproduction and inhalation studies
- Seceded by an IAG with FDA National Center for Toxicological Research for neurotoxicology studies in primates

## Early Efforts

- Joint workshops held with ATSDR and WHO/RIVM to establish human immune testing panels
- Establishment of immunotoxicology questionnaire
- Immunological analyses .....at an ethical narcotics manufacturing facility (*Arch Environ Hlth*, 1995)
- Health & immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment (*Intl Arch Occup Environ Hlth*, 1996)
- Immunological findings among lead-exposed workers (*Amer J Indus Med*, 1998)

## **Highlights of Several Projects**

- Fungal Allergens
- Latex Contact Allergens
- Genetic Risk Factors for Irritant Contact Dermatitis (ICD)

# **NIOSH Fungal Allergen Projects**

- Primary focus is to better understand human exposure and sensitization to fungal bioaerosols.
- Prior to these projects, the paradigm of fungal exposure and sensitization consisted of the inhalation of spores from a select number of species.
- Prevalence and characterization of IgE reactivity to a more expansive fungal panel was explored in several different populations.
- The recent development of fungal detection methods by this group enabled the examination of fungal spores and fragments as sources of aeroallergens.

# West Virginia Fungal Allergic Sensitization

Table 1 SPT reactivity to a panel of common aeroallergens and indoor fungi

Allergen	Number Positive*	Number Negative	Percent Positive	Allergen	Number Positive*	Number Negative	Percent Positive
Perennials				Trees			
Dust mite	40	62	39.2	Box elder	19	83	18.6
Cat	27	75	26.4	Ash	14	88	13.7
Cockroach	22	80	21.5	Elm	14	88	13.7
Dog	7	95	6.8	Birch	12	90	11.7
Mouse	4	98	3.9	Hickory	12	90	11.7
Insects				Oak	12	90	11.7
Caddis fly	16	86	15.6	Sycamore	8	94	7.8
House fly	16	86	15.6	Walnut	8	94	7.8
May fly	14	88	13.7	Beech	6	96	5.8
Moth	10	92	9.8	Juniper	5	97	4.9
Ant	6	96	5.8	Fungi			
Grasses				<i>A. alternata</i>	12	90	11.8
Vernal	27	75	26.4	<i>T. viride</i> #	8	92	8.0
Redtop	26	76	25.4	<i>C. globosum</i> #	7	93	7.0
Timothy	26	76	25.4	<i>P. variotti</i>	7	93	7.0
Fescue	25	77	24.5	<i>A. fumigatus</i>	7	95	6.9
Kentucky Bluegrass	23	79	22.5	<i>C. sphaerospermum</i>	7	95	6.9
Bermuda	20	82	19.6	<i>P. notatum</i>	7	95	6.9
Weeds				<i>A. strictum</i>	6	94	6.0
Short ragweed	27	75	26.4	<i>D. sorokiniana</i>	6	96	5.9
Giant ragweed	24	78	23.5	<i>E. nigrum</i>	5	97	4.9
Plantain	20	82	19.6	<i>C. globosum</i> §	4	96	4.0
Sour dock	18	84	17.6	<i>P. chrysogenum</i>	4	96	4.0
Cocklebur	16	86	15.6	<i>T. viride</i> §	4	96	4.0
Sage brush	16	86	15.6	<i>Pullaria</i> spp.	4	98	3.9
Lambs quarter	12	90	11.7	<i>Rhizopus</i> spp.	4	98	3.9
Kochia	10	92	9.8	<i>S. chartarum</i>	3	97	3.0
Sorrel	7	95	6.8				

\*Values presented represent the total number of individuals (n = 102) identified by SPT to be either positive or negative to a panel of aeroallergen extracts, with the percentage frequency of positive SPTs.

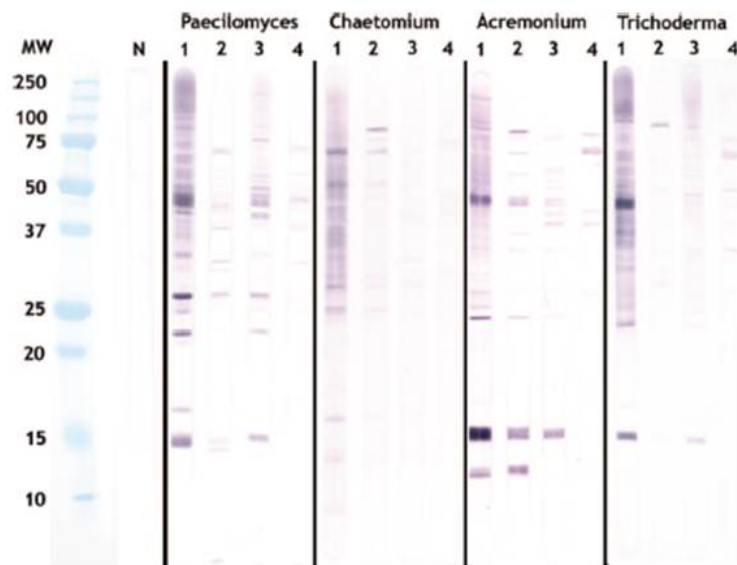
#Extracts of *T. viride* and *C. globosum* prepared commercially by Greer Laboratories.

§Extracts of *T. viride* and *C. globosum* prepared commercially by Antigen laboratories.

The prevalence of fungal sensitization was common, particularly for indoor fungal contaminants that are not routinely included in skin prick test (SPT) panels.

Beezhold *et al. Allergy Asthma Proc* 29: 29-34, 2008

# Characterization of Fungal Allergens



## Cross-reactive allergens in diverse fungal species

Beezhold *et al. Allergy Asthma Proc* 29: 29-34, 2008

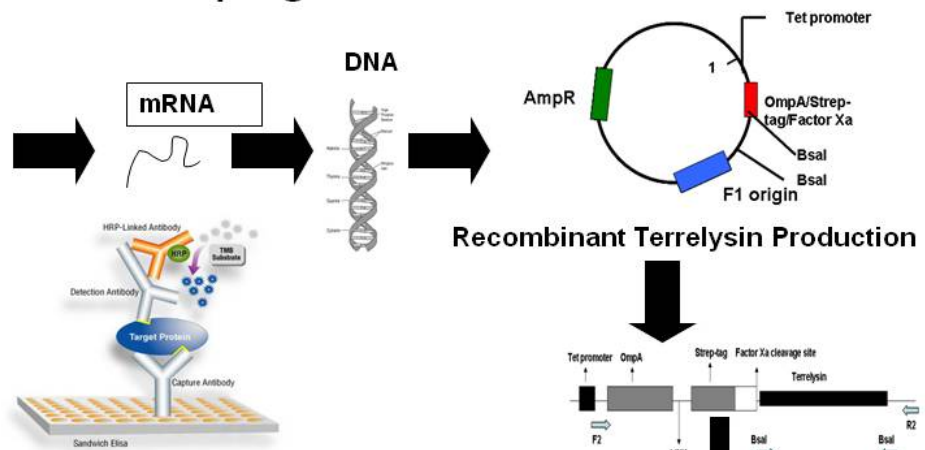
## IgE Specific Reactivity (HEAL Study)

Phadia Immunocap Specific Fungal Allergens	Phadia Immunocap Mx2 Positive Subjects (n=141)		Phadia Immunocap Mx2 Negative Subjects (n=50)	
	Number Positive	Percent Positive	Number Positive	Percent Positive
Penicillium notatum (m1)	100	70.92		0
Cladosporium herbarum (m2)	114	80.85		0
Aspergillus fumigatus (m3)	127	90.07	1	2
Alternaria alternata (m6)	137	97.16		0
Fusarium proliferatum (m9)	122	86.52		0
Epicoccum purpurascens (m14)	134	95.04	1	2
Trichoderma viride (m15)	112	79.43		0
Curvularia lunata (m16)	120	85.11		0
Aspergillus niger (m207)	16	11.35		0
Chaetomium globosum (m208)	26	18.44		0

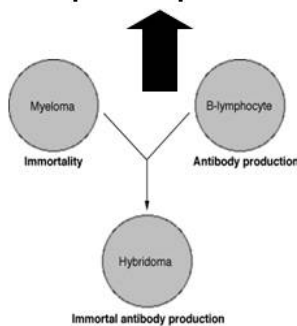
Asthmatic children living post Katrina in New Orleans in highly mold-positive homes prior to remediation

# Development of a Recombinant fungal Hemolysin (Terrelysin) from *Aspergillus terreus*

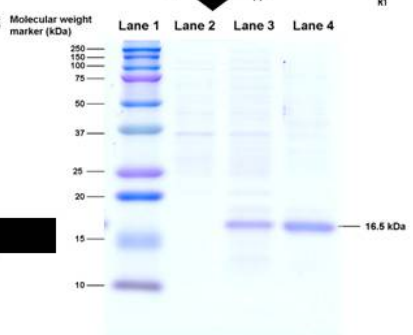
*Aspergillus terreus*



Development of potential clinical and environmental immunoassays



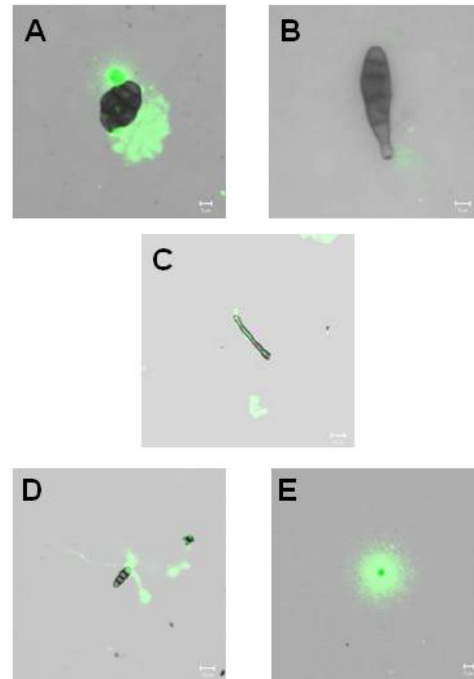
Monoclonal Antibody Production



# Identification of IgE Reactivity using HIA

Confocal fluorescent halogen immunoassay (HIA) immunostaining of fungal spores and hyphal fragments collected from indoor environments in New York City demonstrating IgE binding to expressed antigens using individual patient human serum IgE from a Puerto Rican Cohort.

- (A) Pithomyces
- (B) Alternaria
- (C) Large Hyphal Fragment
- (D) Ascospore
- (E) Aspergillus / Penicillium species.



Indoor fungi as small as 0.5  $\mu\text{m}$  could be immunostained with human IgE

Green B, *et al. Analytical Biochemistry* 354 (1): 151-153, 2006., Green B, *et al. (submitted)*, 2009

**Assay Development  
Identification and Measurement of  
Rubber Contact Allergens**

## **Studies into the Chemical Mechanisms of Latex Contact Allergens**

- Latex and rubber products contain chemical polymerization accelerators that cause allergic contact dermatitis (Type IV allergy)
- Human exposure via medical and industrial gloves, condoms, bandages, surgical drains, dental dams, clothing
- Steps to remove protein (Type 1 allergy) have not decreased contact allergen content

## **American Society for Testing and Materials Standard Method:**

Colorimetric/Spectrophotometric Test Method to Quantify Extractable Chemical Dialkyldithiocarbamate, Thiuram, and Mercaptobenzothiazole Accelerators in Natural Rubber Latex and Nitrile Gloves

- Method is designed to quantify the amount of total extractable accelerators in natural rubber latex (NRL) and nitrile gloves.
- Three common classes of rubber accelerators, the mercaptobenzothiazole (MBT), thiuram, and thiocarbamate type compounds can be detected and quantified by this method based upon differences in binding to cobalt.
- If the specific rubber accelerator(s) present in the glove material is not known, quantification is based on zinc dibutyldithiocarbamate (ZDBC) equivalents.
- Will not detect all potential rubber accelerators, including mercaptobenzothiazole disulfide, dimorpholine, thioureas and diphenyl diamine in which case an HPLC method has been developed.

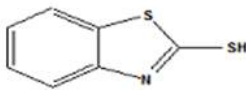


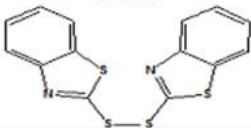


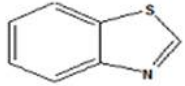


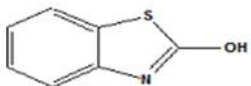


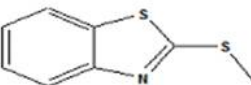


# Survey of Rubber Gloves and Condoms

- Glove Brands Assayed (ND to 7.4 mg accelerator/g glove)
  - 19 Powder Free Latex
  - 5 Powdered Latex
  - 14 Powder Free Nitrile
- Latex Condoms Assayed (ND to 2 mg accelerator/g condom)
  - 14 Brands (lubricated and non-lubricated)
- Results have been published
- Tables of accelerator content in glove brands provided to collaborating dermatologist to help in allergic contact dermatitis (ACD) patient management
- Variety of rubber products associated with patient ACD assayed to assist clinicians in identifying potential causative agent

## **Mechanistic Studies of the Two Most Prevalent Latex Contact Allergen Classes**

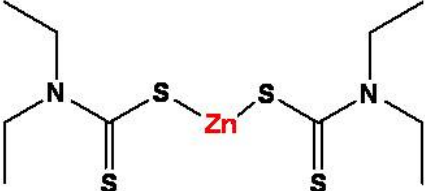
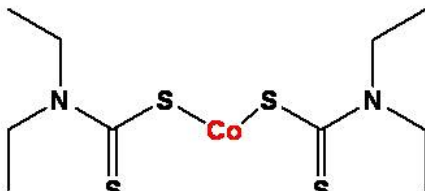
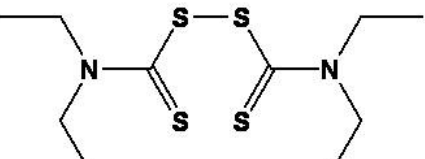
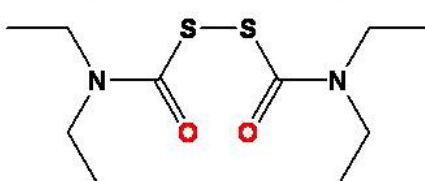
- 2-Mercaptobenzothiazole (MBT)
- Zinc Dithiodialkylcarbamates
  - Zinc diethyldithiocarbamate (ZDEC)
  - Zinc dibutyldithiocarbamate (ZDBC)

### GPMT for Allergic Contact Dermatitis

Challenge Chemical	MBT Sensitized Guinea Pigs		Allergic Reaction
	24 hrs Post Challenge	48 hrs Post Challenge	
<b>MBT</b> 			+++
<b>MBTS</b> 			+++
<b>BT</b> 			-
<b>HBT</b> 			-
<b>MTBT</b> 			-

**Conclusion: MBT allergenicity is mediated through the thiol moiety.**

GPMT = Guinea Pig Maximization Test

Name	Structure	LLNA EC3 Value
zinc diethyldithiocarbamate (ZDEC)		1.01
cobalt diethyldithiocarbamate (CoDEC)		> 7.5
tetraethylthiuram disulfide (TETD)		5.42
tetraethyldicarbonyl disulfide (TEDCD)		1.70

# Approaches to Study Polygenic Diseases

## Candidate gene

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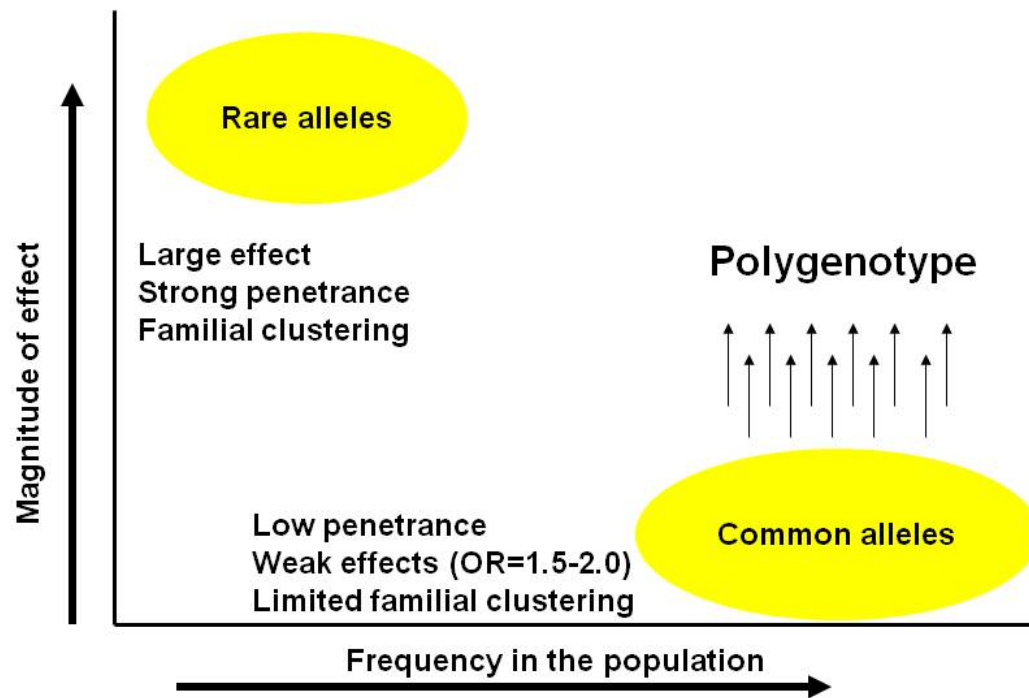
- Biologically plausible, selective, known gene, modest success in complex diseases
  - Hypothesis-driven
  - Lower-cost
  - Fewer false positives

## Genome-wide scan

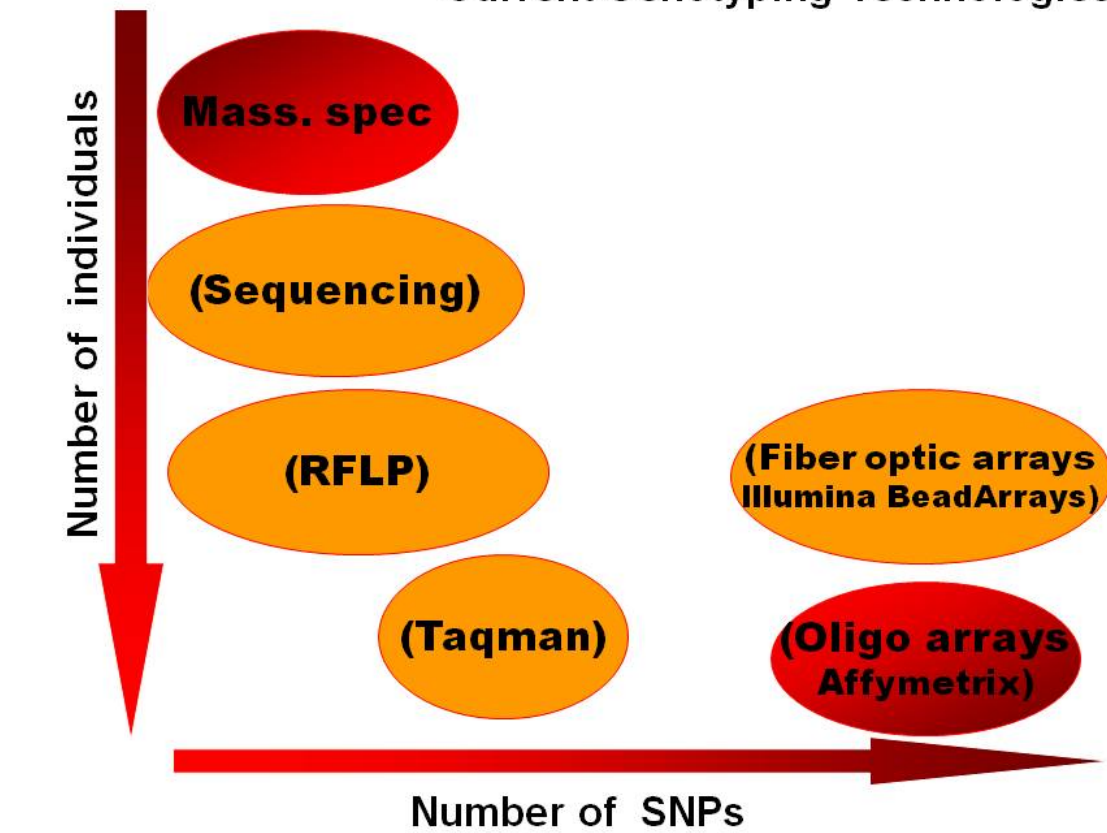
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- Dense set of markers throughout genome because it is not possible to predict all genes or loci that contribute to a disease
  - Hypothesis-free
  - Higher-cost
  - Many false positives

# Common/Rare Variants



## Current Genotyping Technologies



RFLP = restricted fragment length polymorphism  
SNP = single nucleotide polymorphism

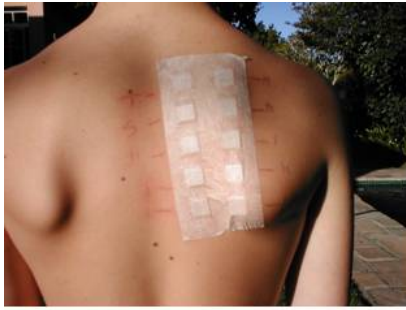
## **Irritant Contact Dermatitis (ICD)**

Peeling red hands from  
too much wet work



# ICD Genetics Study

- Study population consists of 700 health care workers (nurses, physicians, technicians) with no existing skin disease, who wash their hands at least 8 times a day
- Collaborators include dermatologists, statistician, and epidemiologists
- Recruitment sites: Case Western Reserve and West Virginia Universities
- Study duration is approximately 2.5 years



**24 h patch test**  
SLS, BKC, NaOH

## Study Design



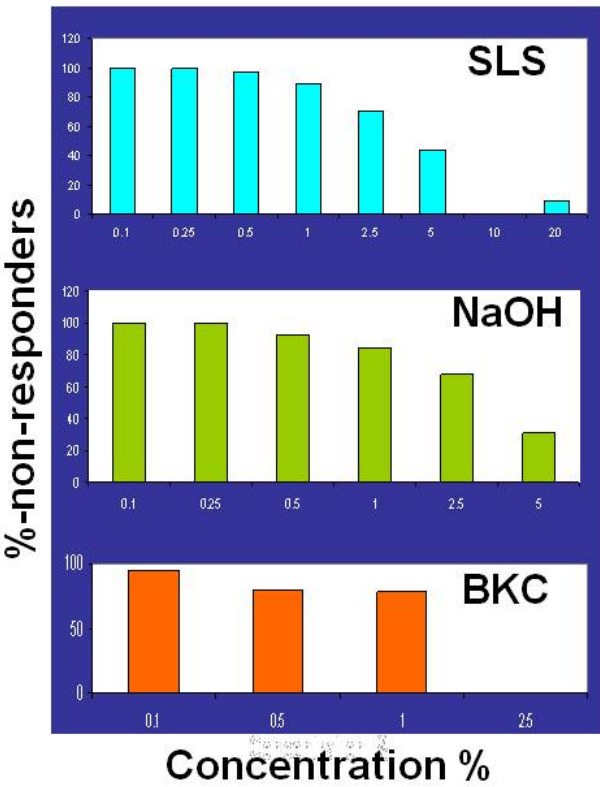
**Transepidermal water loss  
(TEWL) measurement**

### Phase I - pilot study for irritancy threshold/patch test

- Dose range for each of the three irritants ( $EC_{50}$ )
- Assessment of inter- and intra-individual variability
- Irritant threshold – the lowest concentration producing a visible inflammatory response

BKC = benzalkonium chloride  
NaOH = sodium hydroxide  
SLS = sodium lauryl sulfate

# Phase I Feasibility



## Phase II - Patient Evaluations

- Final dose range for each irritant
  - SLS: 2.5%, 5.0% and 20%
  - NaOH: 1%, 2.5% and 5.0%
  - BKC: 0.5%, 1.0% and 2.5%
- Genetic analyses 2 panels:
  - Customized panel
  - Commercial major histocompatibility complex (MHC) panel (~5000 SNPs; 2500 exoncentric and 2500 for mapping panel)
- 6 months follow-up for ICD development
- Association between Patch Test and TEWL results
- Association between SNPs/ ICD development (on-going)

## Some Preliminary Observations

- 63% of volunteers developed hand dermatitis
- Of those with dermatitis, 72% hand washed  $\geq 10$  times/day
- 77% of subjects who reacted to 2.5% SLS and 74% of subjects who reacted to 5% SLS developed dermatitis
  - Whereas non-reacting subjects only developed dermatitis at a rate of 33% and 26%, respectively
- ORs  $> 3.5$  for some glutathione-S-transferase (GST) and tumor necrosis factor alpha ( $\text{TNF}\alpha$ ) variants in dermatitis group
- Frequent hand washing ( $\geq 10$  times/day) may predispose to development of ICD
- Patch testing with SLS and genetic make-up may predict future development of dermatitis and identify sensitive individuals

## **FY 09-10 Projects**

- **Mold studies (D Beezhold):**
  - Characterization of a hemolysin from *Aspergillus terreus*
  - Animal model for airway exposure to dry fungal aerosol
  - Heading off Environmental Asthma in Louisiana (HEAL)
- **Role of genetics environmental/occupational diseases (B Yucesoy):**
  - Chronic Beryllium Disease
  - Allergic contact dermatitis (metals)
  - Occupational asthma
- **Asthma prevalence from disinfectants in the service sector (D Weissman)**

continued

## **FY 09-10 Projects** (continued)

- Allergic sensitization from exposure to indoor air reaction products (Wells & Anderson)
- Determination of Total IgE, antinuclear antibodies and atopy in participants of the Upper Midwest Health Study (R Biagini)
- Antibody levels in SLE (R Biagini)
- Toluene Diisocyanate (TDI)-conjugated protein monoclonal antibodies production and epitope recognition mapping (P Siegel)
- Immune and inflammatory aspects in occupational rhinitis (isocyanates) (V Johnson)

**Questions?**